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A NEW STRAIN OF *S. ALBUS* ISOLATED FROM JABALPUR REGION

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A new strain of *Streptomyces albus* (Isolate No. A-25) was found to be strongly antagonistic to *Fusarium solani* and *Helminthosporium oryzae*.

The antibiotic substance produced by it was found to be thermolabile. It was soluble in n-butanol, methanol and distilled water and showed maximum light absorption in UV range. It differed from Niromycin produced by already known strain of *S. albus* (Waksman and Henrici, 1943). The IR spectrum showed the presence of -OH-, -NH- and C = 0 groups in the antibiotic substances produced by the isolate. The present strain was found to be active against a large number of fungi and did not show any antagonistic activity against bacteria.

During screening of the soil samples collected from geographically different regions of Jabalpur, an Actinomycete isolate A-25 was found to be strongly antagonistic to *Fusarium solani* and *Helminthosporium oryzae*.

The present investigation deals with the taxonomy of the actinomycete isolate A-25. The colour of the growth was recorded on 5th day of incubation. The aerial and substrate mycelium was recorded on 14th day of incubation (Pridham and Lyons, 1961).

Sporophore morphology was studied following the method of Kawato and Shinobu (1959) and Williams and Davis (1967). The shape and surface of spore was studied by Leitz-Dialux 20 microscope. The isolate contains 5-7 spores per chain. The shape of spores was spherical and the size of the spores varied from 1.5 to 1.7 µm.

MATERIALS AND METHODS

After 10 days of incubation in Beef Extract Broth Media at 28°C (±2°C and pH 6.5, the mycelium was filtered out and the filtrate was determined in terms of percentage inhibition of spore germination of *Fusarium solani* and *Helminthosporium oryzae*.

Physical properties of antibiotic

1. *Stability on storage and thermal inactivation point :* The filtrate was distributed in three sets of sterilized and plugged test tubes.

a. In the first set the tubes were stored at temperatures 7°-35°C for 50 days.

b. In the second set the pH was adjusted to varying pH levels.

c. In the third set the tubes were exposed to different temperatures for varying periods. (Table - 1).

Aliquots of the culture filtrate were drawn from time to time and the antibiotic potency was measured as above at room temperature.

2. *Solubility :* The antibiotic substance was extracted from the culture filtrate with n-butanol (Sinha & Sharma, 1984).

3. *Light absorption :* The absorption spectrum of antibiotic substance dissolved in methanol was determined using UV's spectrophotometer 118 by measuring the optical density at different wavelength of light (Fig. 1 a, b) using silica fused photocells and keeping Double Distilled water as Blank. The IR spectrum of the antibiotic substance was recorded on a spectrophotometer Model 1720 X in the range of 4000-500 cm^{-1} by reference to double distilled water. The whole spectrum was caliberated following the standard methods (Dyre, 1978).

4. *Biological activity :* For biological activity Agar Streak Method was used (Waksman and Reilly, 1945). After 5 days incubation at 28°C (±2°C) the inhibition zone if formed was measured in each case.

OBSERVATIONS AND DISCUSSION

The substrate mycelium is light ash, monopodially branched, forming compact growth on media. Aerial mycelium is yellow in colour. Sporophores are open spirals, spores in chains, spherical to oval, 1.5 to 1.7 µm in size as seen under Leitz-Dialux 20 microscope. The organism (Isolate No. A-25) was placed in morphological section-white series in Pridham *et al.* (1958) classification.

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Table 1: Stability of Antibiotic at different temperatures (Percentage Inhibition of Spore Germination)

	0°C		10°C		20°C		30°C		40°C	
Days	F.s.	H.o.	F.s.	H.o.	F.s.	H.o.	F.s.	H.o.	F.s.	H.o.
0	98.33	98.33	98.33	98.33	98.33	98.33	98.33	98.33	98.33	98.33
5	97.32	96.55	98.31	98.00	98.29	98.23	96.05	94.44	97.80	81.82
10	80.59	74.55	89.87	88.68	87.88	70.59	86.49	81.48	86.81	75.71
15	75.17	71.85	77.92	74.23	84.44	59.18	84.54	71.98	84.71	57.89
20	63.46	71.43	73.85	73.21	83.22	58.76	75.20	54.29	76.47	54.55
25	62.04	59.74	72.73	69.01	75.51	57.51	73.93	51.81	63.13	47.22
35.	59.15	44.83	69.94	63.86	70.43	34.63	47.06	41.91	52.95	22.02
40.	54.95	27.40	59.76	48.35	48.23	32.20	42.31	29.55	35.15	10.74
45	54.69	26.42	58.01	38.68	34.56	28.80	40.62	26.25	29.70	7.25
50	47.79	21.85	55.25	37.50	29.60	17.44	28.78	20.41	27.23	6.90

F.s., *Fusarium solani;* H.o., *Helminthosporium oryzae*

Table 2: **The Solubility of antibiotic substance in the selected solvents.**

Solvents	Solubility	*F. solani*	*H. oryza*
Methanol***	+	99.23	98.32
Butanol	+	98.18	98.16
Toluene**	±	98.65	98.63
Pryidine	±	96.95	96.38
Chloroform	±	93.59	96.09
Ethanol	±	96.60	98.47
Dimethyl sulphoxide	±	94.29	87.23
Petroleum ether*	-	91.49	83.78
Benzene	±	92.06	83.06
Acetone	-	92.27	67.09
Distilled water	+	78.97	66.15

*** = Good solubility
** = Moderately soluble
* = Very poor soluble

Table 3: Biological Activity of the antibiotic substances produced by Streptomyces sp. as determined by Agar Streak Method (Waksman & Reilly, 1945).

Fungi	Inhibition zone in mm
Curvularia sp.	21
Trichoderma viride	12
Fusarium solani	11
Aspergillus flavus	16
Aspergillus niger	7
Phoma herbarum	16
Alternaria alternata	10

Biochemical properties :

Positive - Starch hydrolysis, gelatin liquefaction, peptonization of milk, nitrate reduction, H_2S production.

Negative - Tyrosinase reaction, melanin production and cellulolytic activity.

Table 4: Thermal Inactivation Point of antibiotic substance produced by *Streptomyces sp.* (Kept for 30 minutes).

Temperature	% inhibition of spore germination	
in °C	*F. solani*	*H. oryzae*
0	99.79	98.50
10	99.72	98.81
20	99.80	98.69
30	99.89	97.79
40	99.66	97.14
50	89.65	96.12
60	79.58	88.80
70	79.41	88.50
80	79.22	82.37
90	75.50	77.98
100	79.57	60.55
121	56.66	46.55

Carbon utilization :

Positive - glucose, glycerol, mannitol, cellulose, maltose, D-xylose, carboxy methyl cellulose starch & sucrose.

Negative - Fructose, citric acid, oxalic acid.

On the basis of morphological, cultural and some biochemical characteristics the isolate A-25 best resembles *S. albus* (Waksman and Henrici, 1943) However, the latter isolate makes a poor growth on Starch Agar and Yeast Malt Extract Agar media (Pridham and Tresner, 1974; Waksman, 1961; Shirling and Gottlieb, 1969) where as the present isolate A-25 grows luxuriantly in both of these media. In the latter medium the aerial mycelium is creamish yellow in case of *S. albus* whereas it is whitish grey in isolate A-25.

The isolate A-25 and *S. albus* differ in their carbon utilization spectra.

Carbon sources	*S. albus*	Isolate A-25
1. D-glucose	+	+
2. D-Xylose	+	+
3. Mannitol	+	+
4. Fructose	+	Variation
5. Sucrose	-	+

It is therefore justified to regard the isolate A-25 as a new strain of *S. albus* because both the cultures though different in some cultural characters as well as in carbon utilization resemble each other in sporophore morphology.

Properties of antibiotic substance :

The antibiotic substance is thermolabile (Table -4) The antibiotic activity of the culture filtrate decreases with the increase in storage time at all temperatures. The loss of activity was relatively less at lower than at higher temperatures. The filtrate retained its original potency up to 30th day when stored at 0°C pH-6.5, 7.5 and 8.5 Alkaline as well as acidic pH ranges do not favour stability of the active principle. The most suitable pH & temperature for storage of the active substance are 7.5 at 10°C.

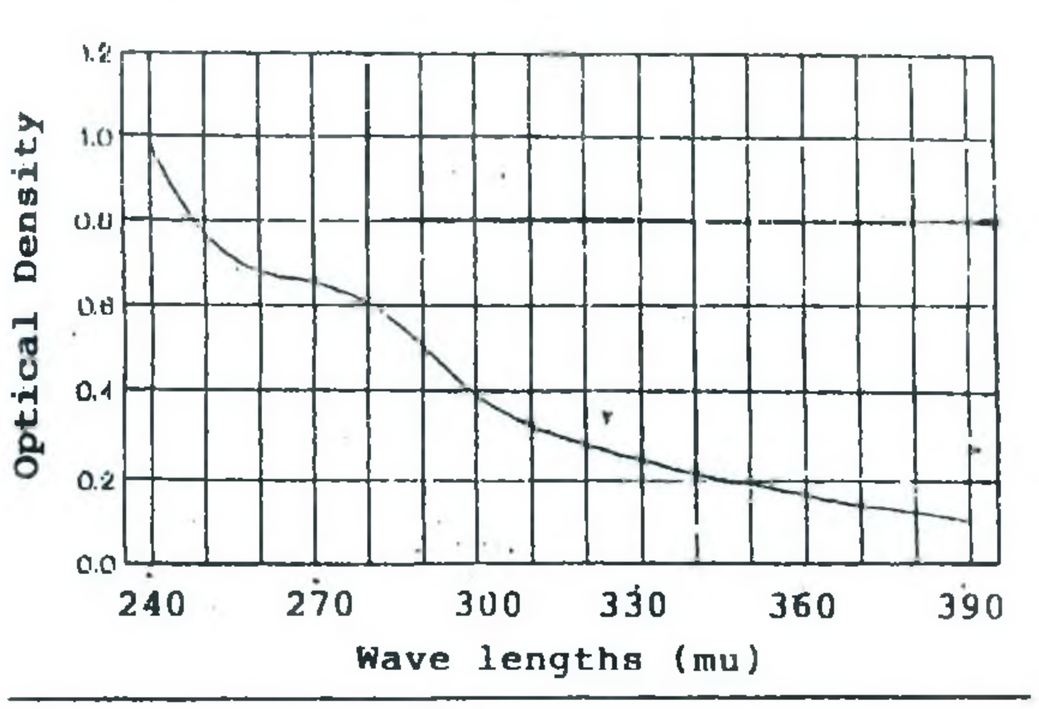
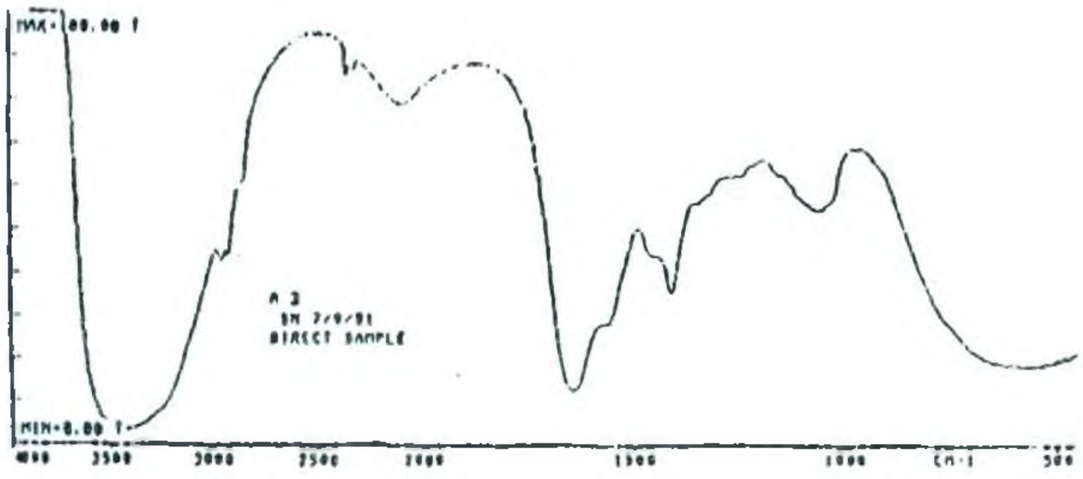


Fig 1a



Absorption curve of antibiotic substance in IR range.

(Fig 1b)

Figures 1a,b. Showing optical density; and absorption spectrum of antibiotic

The antibiotic reduces its inhibitory property with the increasing temperature as well as duration when kept at 90°C for 30 minutes. The same results were also found by Pal and Nandi (1964), Batra and Bajaj (1966), Sinha and Basuchaudhary (1977).

The culture filtrate was diluted 10 times but still the original activity of antibiotic was not so much effected.

The antibiotic substance was best soluble in methanol, butanol and ethanol exhibited good activity where as in chloroform, benzene, toluene, pyridine, dimethyl-sulphoxide, the solubility was reduced and the percentage inhibition of spore germination was also reduced. It was sparingly soluble in petroleum ether but in acetone almost negotiable (Table - 2).

The antibiotic substance present in culture filtrate is active against a large number of micro-organisms some of them being important plant and human pathogens. Hence there is a possibility of its use in control of some important plant and human diseases (Table-3).

The absorption maximum is characteristic of some non-polyenic antibiotic for which absorption maximum has been reported (Waksman and Lechevalier, 1962). The antibiotic substance differs from niromycin produced by already known strains of *S. albus* and also related antibiotics such as fermicidin and cycloheximide produced by *Streptomyces sp.* similar to *S. griseolus* because they are not active against bacteria. The IR spectrum of the antibiotic substance under study shows a broad peak in 3500-3000 cm^{-1} and the significant absorption at 1600, 1550 and 1450-1350 cm^{-1} indicates the presence of C=O, N-H bonding.

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